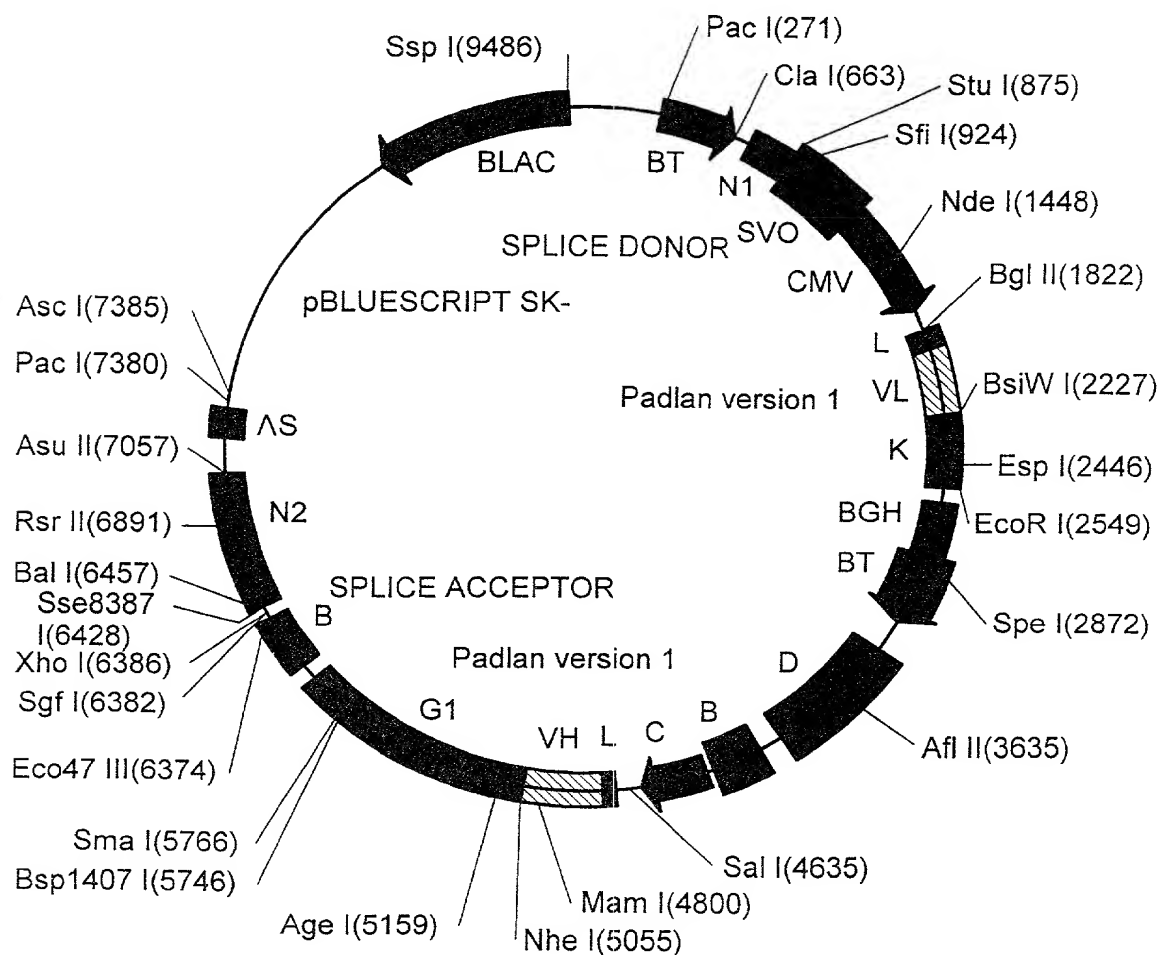


# FIG. 1

## Anti-GP39 HU24-31 in N5KG1 (Version #1)



9597 BP

Map BY Mitchell Raff

06/01/95

C = Cytomegalovirus promoter/enhancer

BT = Mouse Beta globin major promoter

B = Bovine growth hormone polyadenylation

N1 = Neomycin phosphotransferase exon 1

N2 = Neomycin phosphotransferase exon 2

K = Human immunoglobulin kappa constant region

G1 = Human immunoglobulin gamma 1 constant region

VL = Anti-GP39 variable light region (version #1)

VH = Anti-GP39 variable heavy region (version #1)

NONCUTTERS = AvrII, BstI 107I, DraIII, FseI, HindIII, I-PpoI, I-SceI,

KpnI, MluI, MunI, PmeI, PmlI, SgrAI, SrfI, Swa I, XbaI, XcmI

N5KG1 cut BglII + BsiWI and VL dropped in & cut SalI + NheI and VH dropped in.

L = Leader

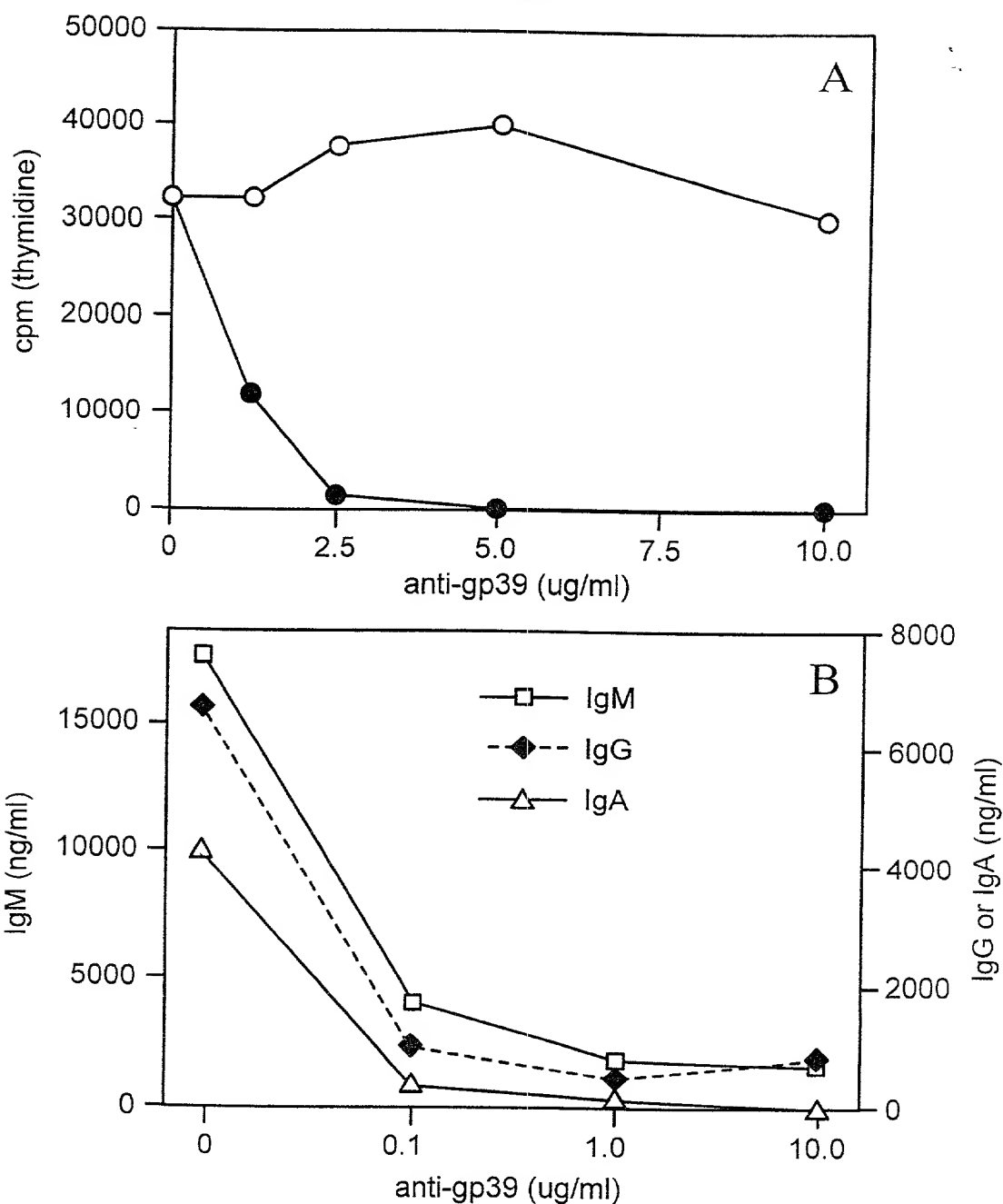
SVO = SV40 origin

SV = SV40 polyadenylation

D = Dihydrofolate Reductase

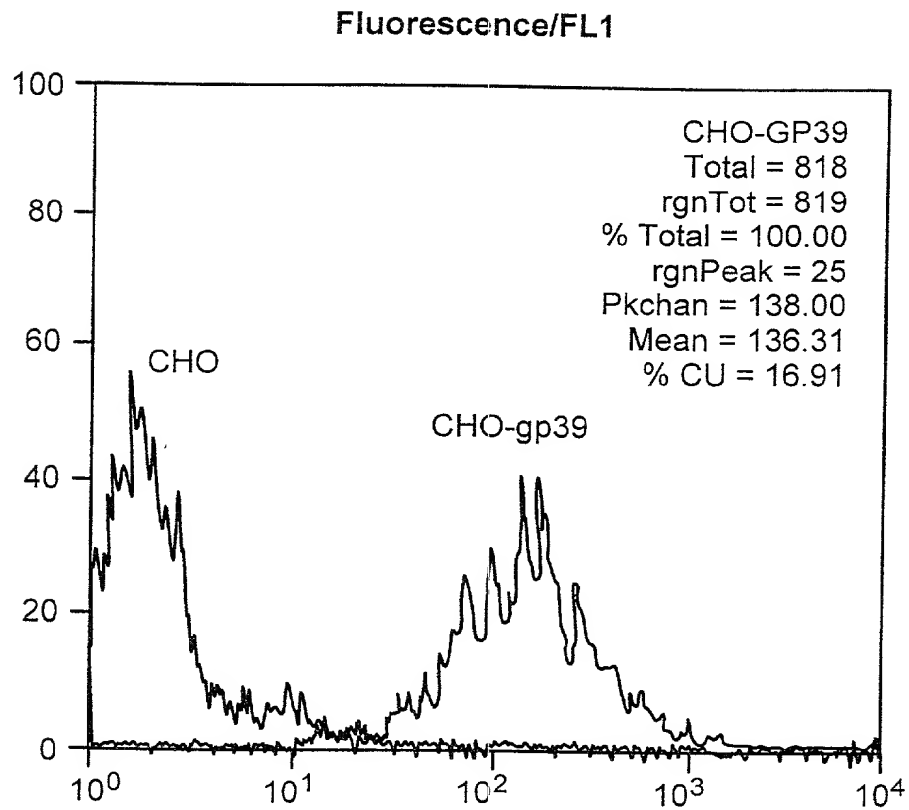
BLAC = Betalactamase gene

# FIG. 2



Anti-gp39 inhibits B cell proliferation and differentiation, but not allogeneic T cell proliferation. A. Human PBL were cultured in 96 well plates ( $0.1 \times 10^6$ /well) in the presence or absence of the 20% (v/v) soluble gp39-CD8 (sgp39-CD8) fusion protein and 5 ug/ml rhIL-4 for 3 d. Anti-gp39 mAb, 24-31 (●), or a control murine IgG1 mAb (○), were added at a range of concentrations (1.25-10 ug/ml). Cultures were pulsed with 1  $\mu$ Ci  $^3$ H-thymidine during the final 6 hr of a 72 hr culture period. B. Mitomycin treated T cells ( $5 \times 10^4$ /well) activated with immobilized anti-CD3 (64.1) were cultured with  $2.5 \times 10^4$ /well IgD<sup>+</sup> B cells in 96-well microtiter plates for 12d in the presence or absence of various concentrations (0.1-10.0 ug/ml) of anti-gp39 mAb, 24-31. Culture supernatants were subsequently assayed for IgM (□), IgG (◆), and IgA (△) by isotype specific ELISA.

**FIG. 3**



FACS analysis of non-transfected CHO cells and a gp39 transfectant.  $1 \times 10^6$  cells were treated with the mouse anti-gp39 antibody 24-31 and then with a goat-anti-mouse IgG-FITC conjugate (Southern Biotechnology Associates). The samples were analyzed on FACScan (Becton Dickinson).

24-31 Humanized V<sub>L</sub> #1

5'	<u>BglIII</u>				9					18					27					36					45					54	
	AGA	TCT	CTC	ACC		ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC												
						M	G	F	K	M	E	S	Q	F	L	A	F	V	F												
					63					72					81					FR1	90					99					108
	GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CCA													
	A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	P													
					117					126					135					144					153	CDR1				162	
	TCT	TTC	CTC	TCC	GCC	TCC	GTA	GGA	GAC	AGG	GTC	ACC	ATC	ACC	TGC	AAG	GCC	AGT													
	S	F	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S													
					171					180					189	FR2				198					207					216	
	CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	AAG	TCT	CCT													
	Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	K	S	P													
					225					234	CDR2				243					252	FR3				261					270	
	AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC													
	K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F													
					279					288					297					306					315					324	
	TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	TCT	CTC	CAG	CCA													
	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P													
					333					342					351	CDR3				360					369					378	
	GAA	GAC	TTC	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC													
	E	D	F	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F													
	FR4				387					396					405	<u>BsiWI</u>															
	GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA	CGT	ACG																				
	G	G	G	T	K	L	E	I	K	R	T																				

FIGURE 4

24-31 Humanized V<sub>L</sub> #2

5'	<u>BglII</u>				9				18				27				36				45				54			
	AGA	TCT	CTC	ACC	ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC										
					M	G	F	K	M	E	S	Q	F	L	A	F	V	F										
	63				72				81				90				99				108				FR1			
	GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CCA										
	A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	P										
	117				126				135				144				153				CDR1				162			
	GAT	TCT	CTC	GCC	GTG	TCC	CTC	GGA	GAG	AGG	GCC	ACC	ATC	AAC	TGC	AAG	GCC	AGT										
	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	K	A	S										
	171				180				189				FR2				198				207				216			
	CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	CAA	TCT	CCT										
	Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	Q	S	P										
	225				234				CDR2				243				252				FR3				261			
	AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC										
	K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F										
	279				288				297				306				315				324				333			
	TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	TCT	CTC	CAG	GCC										
	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	A										
	333				342				351				CDR3				360				369				378			
	GAA	GAC	GTG	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC										
	E	D	V	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F										
	FR4				387				396				405				<u>BsiWI</u>				3'							
	GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA	CGT	ACG																	
	G	G	G	T	K	L	E	I	K	R	T																	

FIGURE 5

24-31 Humanized V<sub>H</sub> #1

5'	SalI		9			18			27			36			45			54		
	GTC	GAC	ATG	ATG	GTG	TTA	AGT	CTT	CTG	TAC	CTG	TTG	ACA	GCC	CTT	CCG	GGT	TTC		
			M	M	V	L	S	L	L	Y	L	L	T	A	L	P	G	F		
	CTG	TCA	63 FR1			72			81			90			99			108		
	---	---	GAG	GTG	CAG	CTT	CAG	GAG	TCA	GGA	CCT	GGC	CTC	GTG	AAA	CCT	TCT	GAG		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	L	S	E	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	E		
	ACT	CTG	117			126			135			144			153 CDR1			162		
	---	---	TCC	CTC	ACC	TGT	ACC	GTC	TCT	GGC	GAC	TCC	ATC	ACT	AAT	GGT	TTC	TGG		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	T	L	S	L	T	C	T	V	S	G	D	S	I	T	N	G	F	W		
	ATC	TGG	171 FR2			180			189			198			207 CDR2			216		
	---	---	ATC	CGG	AAA	CCA	CCA	GGG	AAT	AAA	CTT	GAG	TAC	ATG	GGC	TAC	ATA	AGT		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	I	W	I	R	K	P	P	G	N	K	L	E	Y	M	G	Y	I	S		
	TAC	AGT	225			234			243			252			261 FR3			270		
	---	---	GGT	AGC	ACT	TAC	TAC	AAT	CCA	TCT	CTC	AAG	AGT	CGA	ATC	TCC	ATC	TCT		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	Y	S	G	S	T	Y	Y	N	P	S	L	K	S	R	I	S	I	S		
	CGC	GAC	279			288			297			306			315			324		
	---	---	ACA	TCC	AAG	AAC	CAG	TTC	TCT	CTA	AAG	TTG	TCT	TCT	GTG	ACT	GCC	GCC		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	R	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A		
	GAC	ACA	333			342			351 CDR3			360			369			378		
	---	---	GGC	GTG	TAT	TAC	TGT	GCC	TGC	CGC	AGT	TAC	GGG	AGG	ACC	CCG	TAC	TAC		
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	D	T	G	V	Y	Y	C	A	C	R	S	Y	G	R	T	P	Y	Y		
	TTT	GAC	387			396			405			414			423 NheI			3'		
	---	---	TTC	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACC	GTC	TCC	TCA	GCT	AGC				
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
	F	D	F	W	G	Q	G	T	T	L	T	V	S	S	A	S				

FIGURE 6

# Anti-gp39 24-31 V<sub>K</sub> Sequence

5'	<u>Bgl II</u>			9			18			27			36			45			54					
	AGA	TCT	CTC	ACC	ATG	GGC	TTC	AAG	ATG	GAG	TCA	CAG	TTT	CTG	GCC	TTT	GTA	TTC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
				M	G	F	K	M	E	S	Q	F	L	A	F	V	F							
	63			72			81			+1			90			FRI			99			108		
	GCG	TTT	CTC	TGG	TTG	TCT	GGT	GTT	GAT	GGA	GAC	ATT	GTG	ATG	ACC	CAG	TCT	CAA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	A	F	L	W	L	S	G	V	D	G	D	I	V	M	T	Q	S	Q						
	117			126			135			144			153			CDR1			162					
	AAA	TTC	ATG	TCC	ACA	TCC	GTA	GGA	GAC	AGG	GTC	AGC	ATC	ACC	TGC	AAG	GCC	AGT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	K	F	M	S	T	S	V	G	D	R	V	S	I	T	C	K	A	S						
	171			180			189			FR2			198			207			216					
	CAG	AAT	GTG	ATT	ACT	GCT	GTA	GCC	TGG	TAT	CAA	CAG	AAA	CCA	GGA	CAA	TCT	CCT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	Q	N	V	I	T	A	V	A	W	Y	Q	Q	K	P	G	Q	S	P						
	225			234			CDR2			243			252			FR3			261			270		
	AAA	TTG	CTG	ATT	TAC	TCG	GCA	TCC	AAT	CGG	TAC	ACT	GGA	GTC	CCT	GAT	CGC	TTC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	K	L	L	I	Y	S	A	S	N	R	Y	T	G	V	P	D	R	F						
	279			288			297			306			315			324								
	TCA	GGC	AGT	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC	ATC	AGC	AAT	ATG	CAG	TCT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	S	G	S	G	S	G	T	D	F	T	L	T	I	S	N	M	Q	S						
	333			342			351			CDR3			360			369			378					
	GAA	GAC	CTG	GCA	GAT	TAT	TTC	TGC	CAG	CAA	TAT	AAC	AGC	TAT	CCG	TAC	ACG	TTC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	E	D	L	A	D	Y	F	C	Q	Q	Y	N	S	Y	P	Y	T	F						
	FR4			387			396			405			<u>Bsi WI</u>			3'								
	GGA	GGG	GGG	ACC	AAG	CTG	GAA	ATC	AAA	CGT	ACG													
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	G	G	G	T	K	L	E	I	K	R	T													

FIGURE 7

Anti gp39 24-31 V<sub>H</sub> Sequence

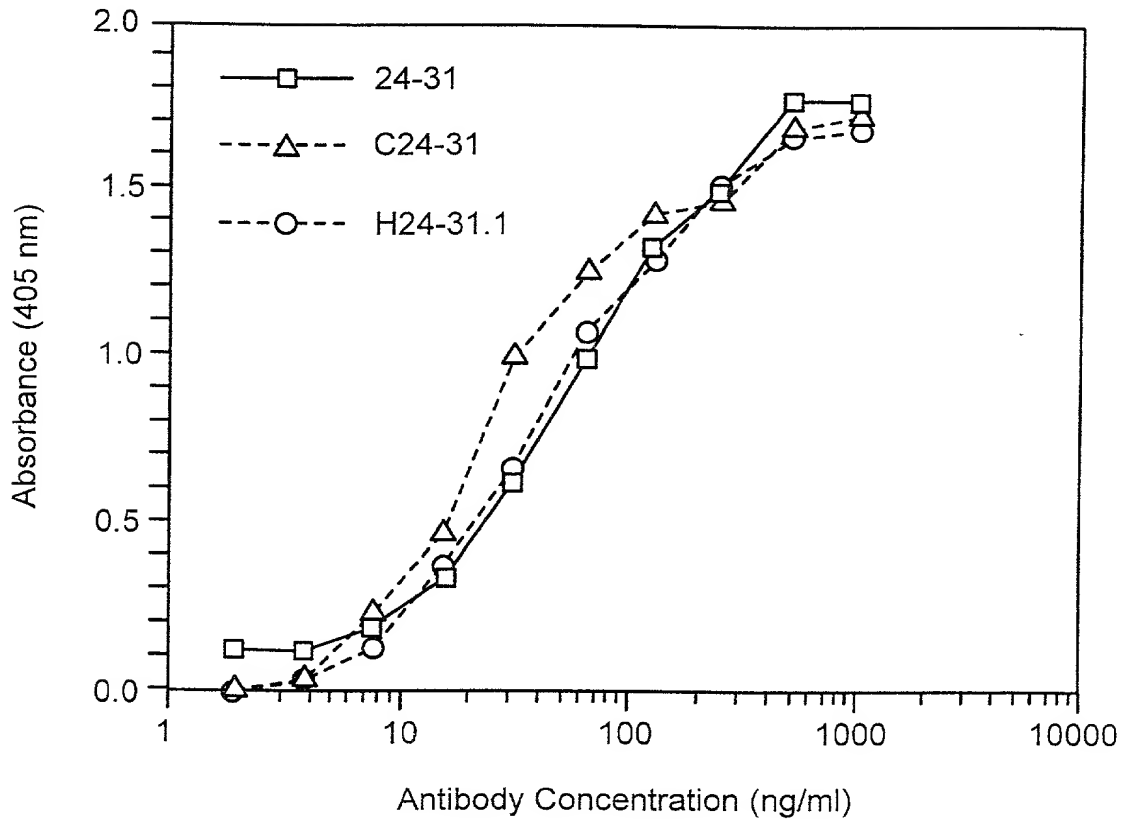
SalI		9		18		27		36		45		54						
5'	GTC	GAC	ATG	ATG	GTG	TTA	AGT	CTT	CTG	TAC	CTG	TTG	ACA	GCC	CTT	CCG	GGT	TTC
			M	M	V	L	S	L	L	Y	L	L	T	A	L	P	G	F
			+1															
	CTG	TCA	GAG	GTG	CAG	CTT	CAG	GAG	TCA	GGA	CCT	AGC	CTC	GTG	AAA	CCT	TCT	CAG
	L	S	E	V	Q	L	Q	E	S	G	P	S	L	V	K	P	S	Q
			117			126			135			144			153	CDR1	162	
	ACT	CTG	TCC	CTC	ACC	TGT	TCT	GTC	ACT	GGC	GAC	TCC	ATC	ACT	AAT	GGT	TTC	TGG
	T	L	S	L	T	C	S	V	T	G	D	S	I	T	N	G	F	W
			171	FR2		180			189			198			207	CDR2	216	
	ATC	TGG	ATC	CGG	AAA	TTC	CCA	GGG	AAT	AAA	CTT	GAG	TAC	ATG	GGC	TAC	ATA	AGT
	I	W	I	R	K	F	P	G	N	K	L	E	Y	M	G	Y	I	S
			225			234			243			252			261	FR3	270	
	TAC	AGT	GGT	AGC	ACT	TAC	TAC	AAT	CCA	TCT	CTC	AAG	AGT	CGA	ATC	TCC	ATC	ACT
	Y	S	G	S	T	Y	Y	N	P	S	L	K	S	R	I	S	I	T
			279			288			297			306			315			324
	CGC	GAC	ACA	TCC	CAG	AAC	CAG	TTC	TAC	CTA	CAA	TTG	AAT	TCT	GTG	ACT	ACT	GAG
	R	D	T	S	Q	N	Q	F	Y	L	Q	L	N	S	V	T	T	E
			333			342			351	CDR3		360			369			378
	GAC	ACA	GGC	ACA	TAT	TAC	TGT	GCC	TGC	CGC	AGT	TAC	GGG	AGG	ACC	CCG	TAC	TAC
	D	T	G	T	Y	Y	C	A	C	R	S	Y	G	R	T	P	Y	Y
			387	FR4		396			405			414			423	NheI		
	TTT	GAC	TTC	TGG	GGC	CAA	GGC	ACC	ACT	CTC	ACC	GTC	TCC	TCA	GCT	AGC	3'	
	F	D	F	W	G	Q	G	T	T	L	T	V	S	S	A	S		

FIGURE 8



**FIG. 9**

**Direct Binding of Anti-gp39 Antibodies to mgp39 CHO Cells**

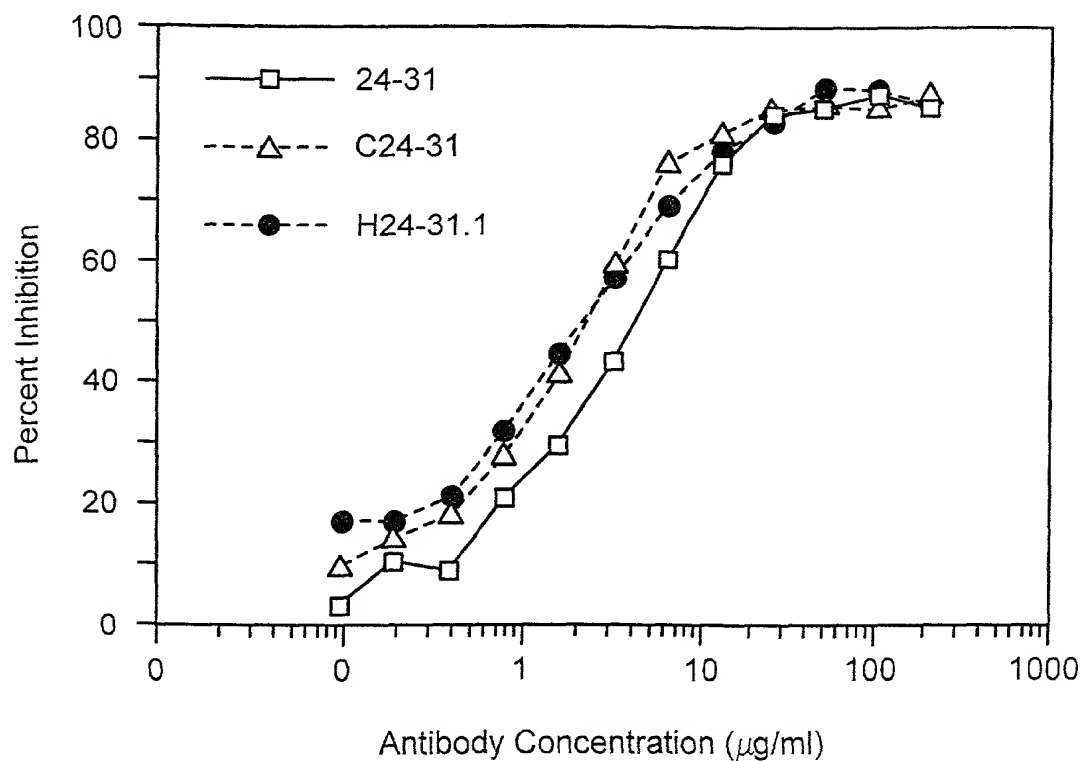


Fifty  $\mu$ l of 1  $\mu$ g/ml solution of each anti-gp39 antibody (murine, chimeric and humanized version 1 of 24-31) was added to wells containing poly-l-lysine fixed mgp39 CHO cells. After a 2 hour incubation, the bound antibodies were detected with either goat anti-human IgG HRP or goat anti-mouse IgG HRP. The binding capacity of each antibody was compared on a plot of absorbance vs antibody concentration.

The figure shows that the half maximal binding in ELISA is achieved at similar concentrations for all three versions, at approximately 40 ng/ml.

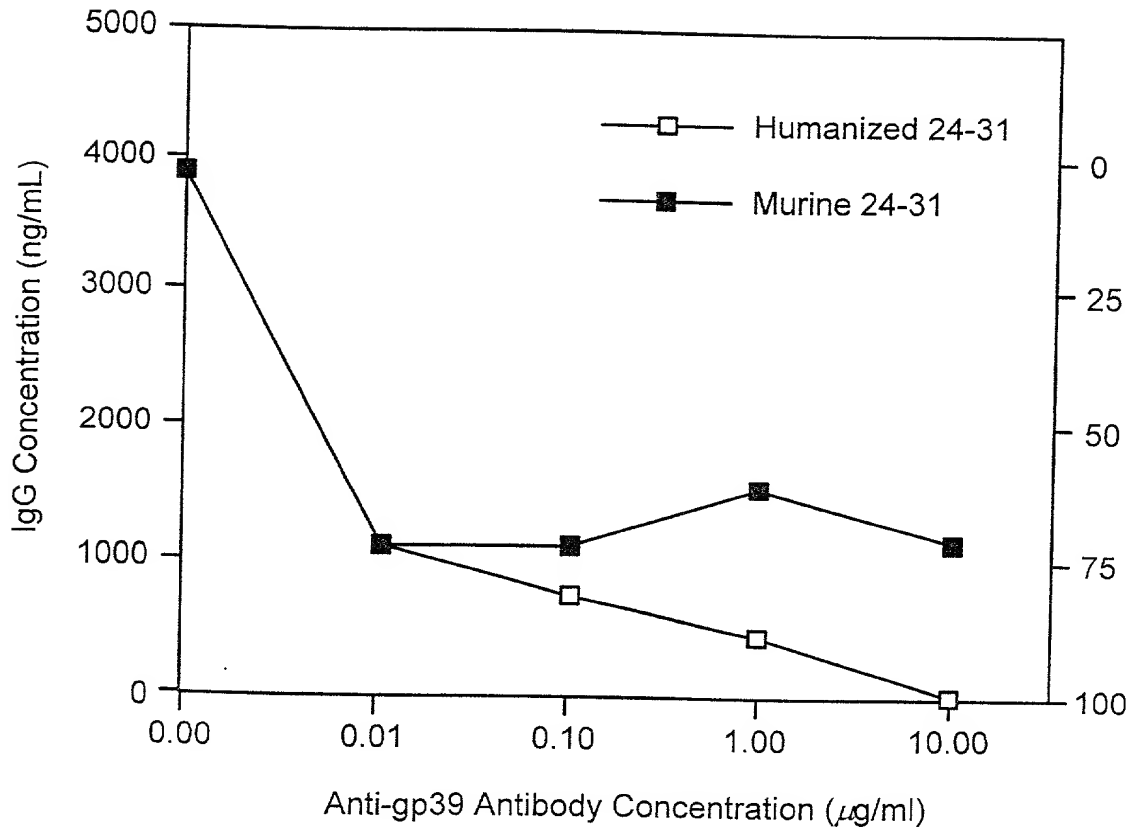
**FIG. 10**

**Competition Binding of 200 ng/ml Mouse Anti-gp39 Biotin  
with Anti-gp39 Antibodies on mgp39 CHO Cells**



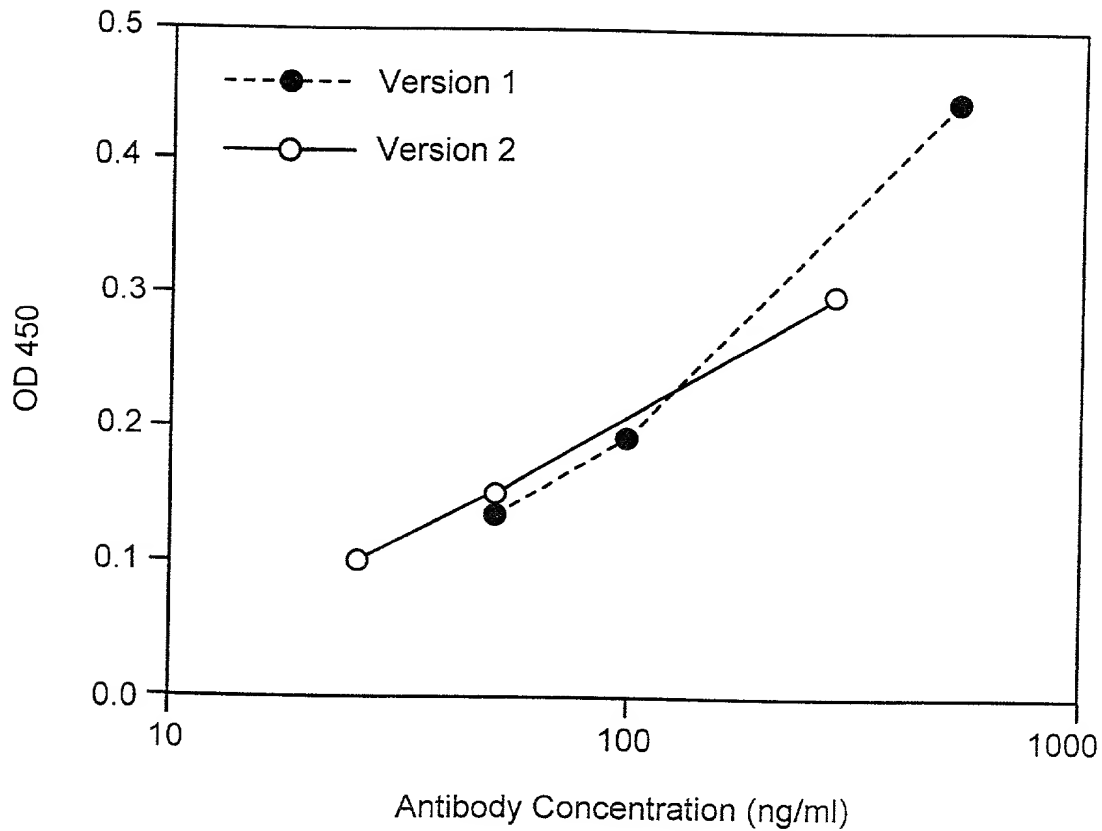
The figure shows that competition for binding to gp39 between biotin label 24-31 and the mouse, chimeric and humanized versions 1 are comparable, possibly with the humanized version slightly better than the original antibody, with half-maximal competition at 2 and 4  $\mu\text{g/ml}$ , respectively.

**FIG. 11**



Purified, mitomycin C treated T cells were added into cell culture plates coated with anti-CD3 antibody. Autologous purified B cells were mixed with antibody at described concentrations and added to these plates in regular growth media. After 10 days the supernatant was tested for content of human IgM.

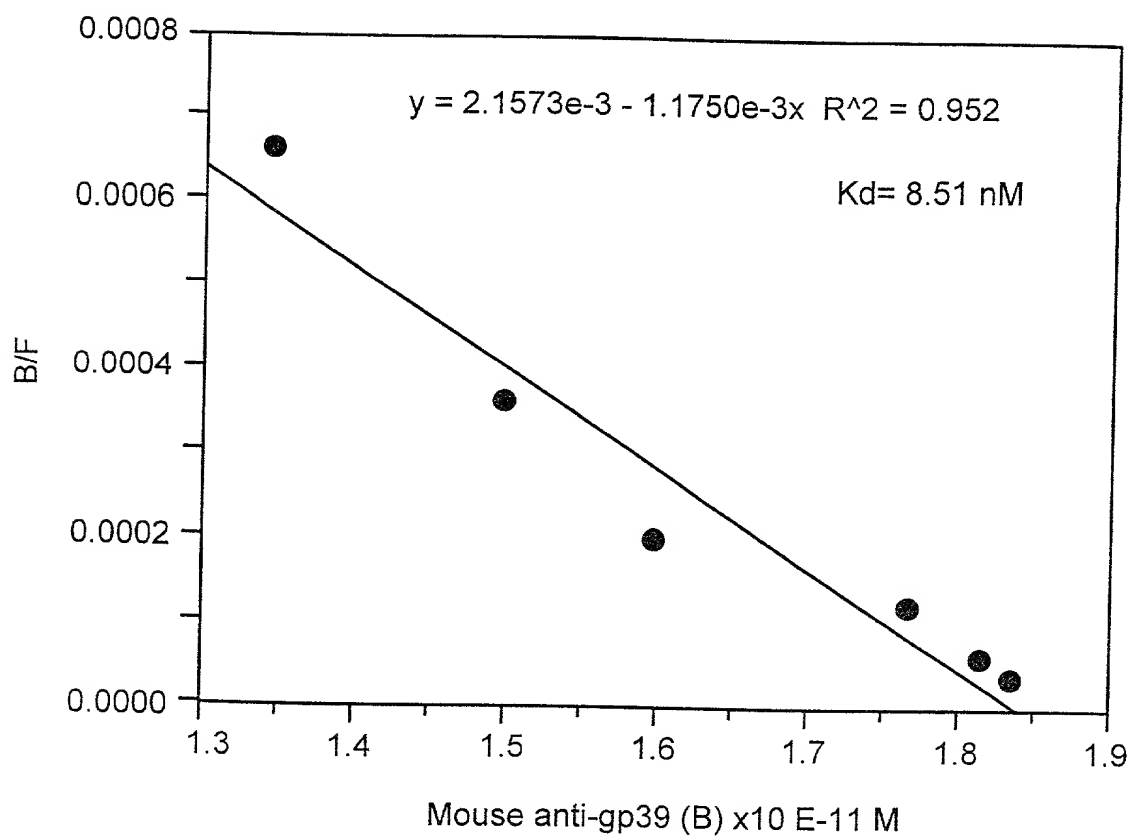
**FIG. 12**



CHO cell supernatant containing humanized 24-31 version 1 and version 2 in unknown amounts, was incubated on mgp39-CHO cells for 2 hours. After a wash, the amount of bound antibody was determined. The same supernatants were tested in parallel on an ELISA plate coated with Goat  $\alpha$  Human  $\gamma$ , to determine the concentration of human IgG present relative to a control of known concentration. The binding data were normalized relative to the total antibody concentration.

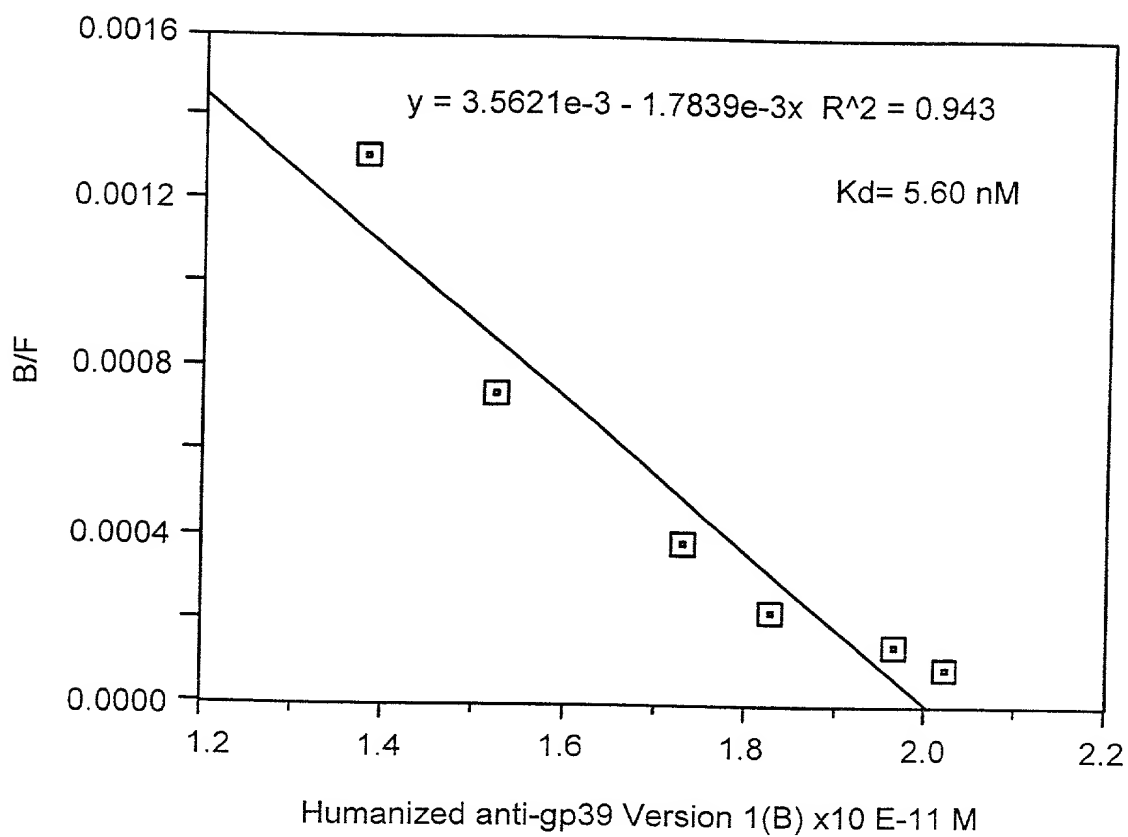
**FIG. 13**

**Scatchard Analysis**



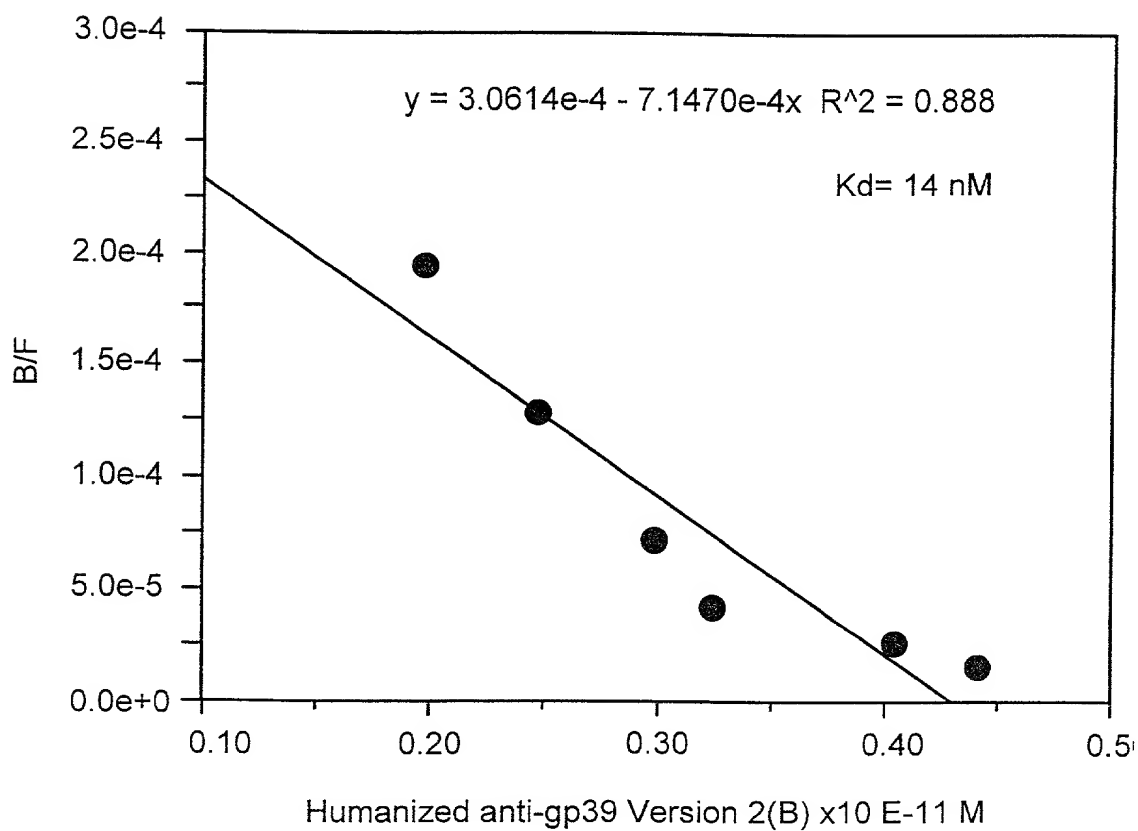
# FIG. 14

## Scatchard Analysis



**FIG. 15**

**Scatchard Analysis**



Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-2 Cytokine Release from CD4+ T Cells in Presence of Immobilized Anti-CD3

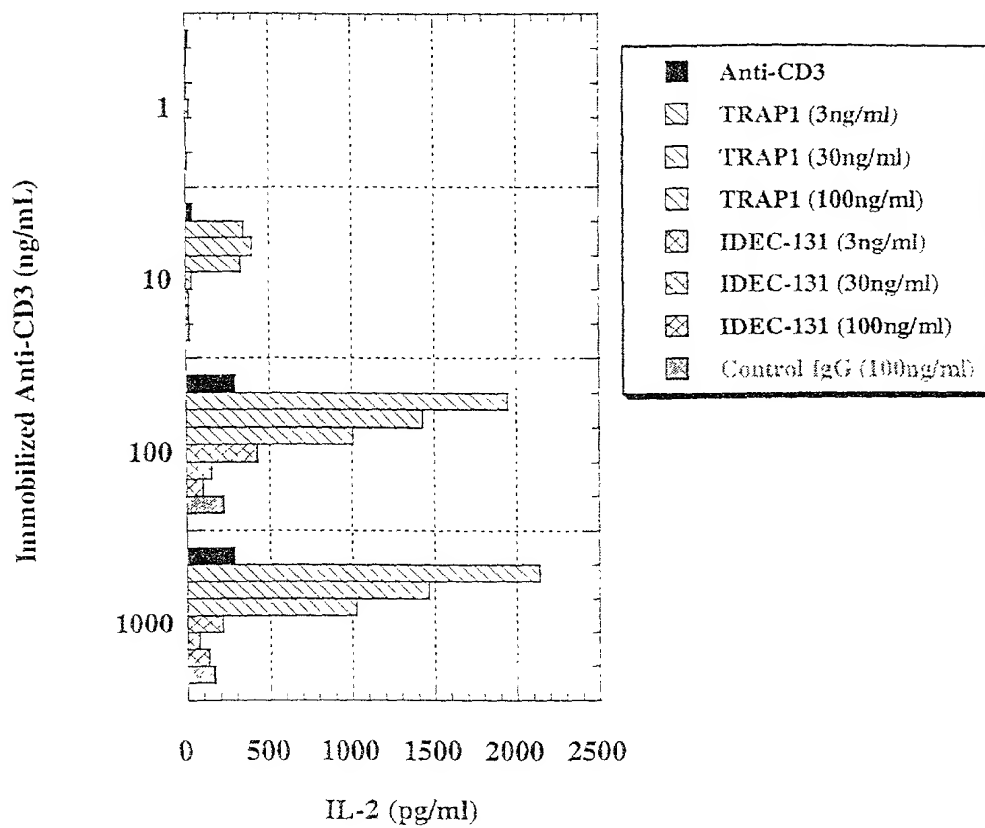


Figure 16



Soluble Anti-CD40L Antibody (TRAP1) Stimulates IL-4 Cytokine Release from CD4+ T cells in Presence of Immobilized Anti-CD3

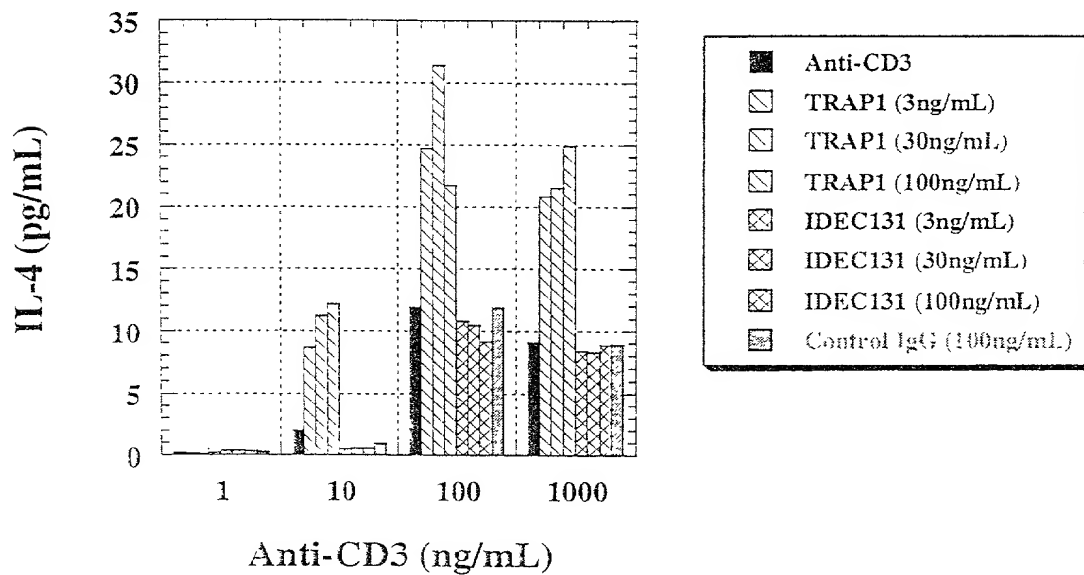


Figure 17

Stimulation of IL-2 by Agonist TRAP1 Anti-CD40L is Dependent  
on Co-stimulation with Anti-CD3 and Signaling Through CD40L

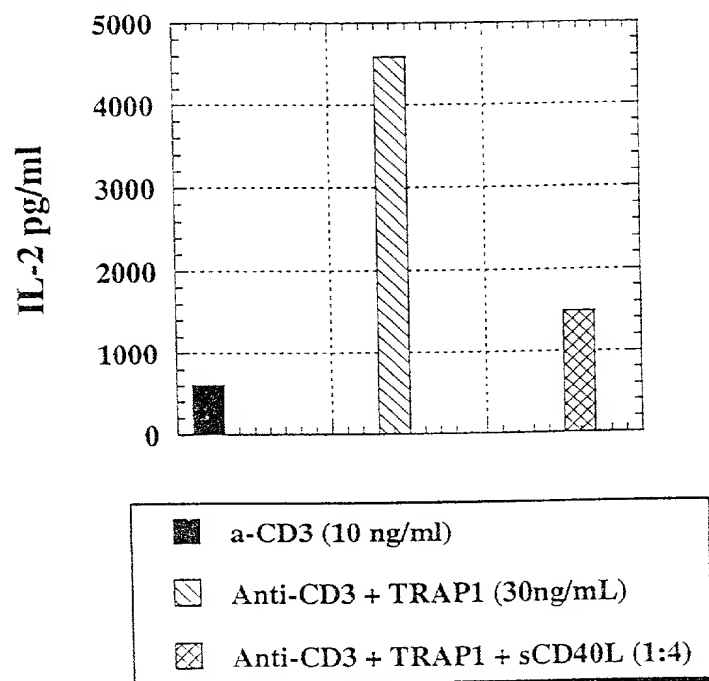


Figure 18

109030 " 444/360

Soluble Anti-CD40L Antibody (TRAP1) Stimulates Gamma Interferon Release from CD4<sup>+</sup> T cells in Presence of Immobilized Anti-CD3

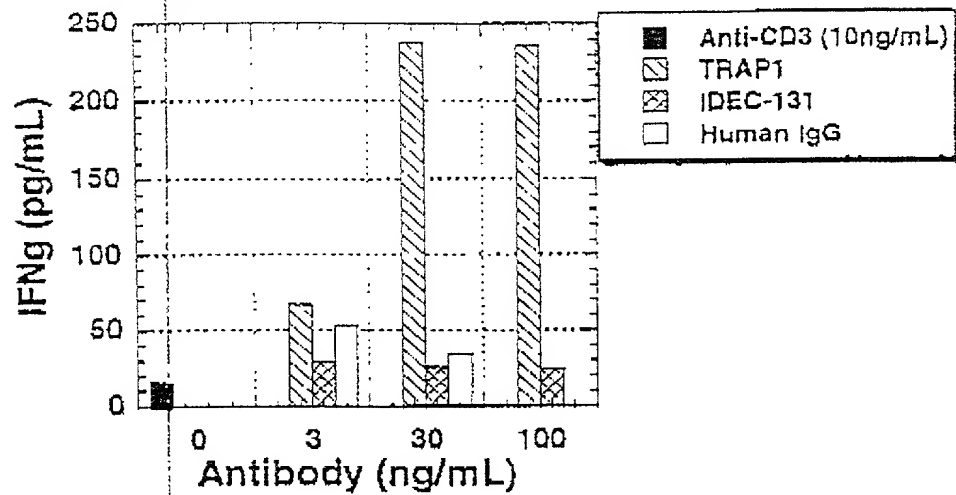


Figure 19

Effect of Anti-CD40L Monoclonal Antibodies on Proliferation of Human CD4<sup>+</sup> T cells in vitro

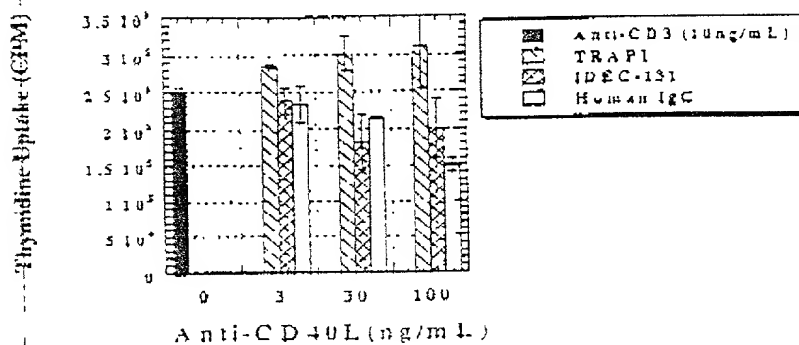


Figure 20